

## VIRUS-RESISTANCE GENES: THE MOUSE MODEL.

### GENES DE RESISTÊNCIA VIRAL: O MODELO MURINO.

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**Abstract:** Human and animals differ widely in their responses to virus infections. Viruses may induce barely discernible symptoms in some of them and severe, life-threatening illness in others.

Evidence is accumulating that genetic background is one of the major factors involved, thus, identifying genes that control the response to virus infection is a crucial step in elucidating how they might affect the pathophysiological processes underlying the severity of the disease induced.

In this review, it's illustrated how mouse-virus systems are being used to identify candidate virus-resistance genes, and how they provide the probes to detect functional homologues of resistance genes that are shared by rodent and other species.

**Resumo:** Os Homens e os animais diferem grandemente nas suas respostas às infecções virais. Os vírus podem induzir, em alguns, sintomas ligeiros, enquanto que em outros podem provocar patologias graves mesmo mortais.

Acumulam-se evidências que o património genético é um dos factores primordiais a condicionar e contribuir para a complexidade das interações vírus-hospedeiro. A identificação de genes com papel na resposta à infecção viral tornou-se pois o tema de investigação de muitos laboratórios, com o objectivo de elucidar os processos fisiopatológicos que regem e determinam esse tipo de resposta.

Neste artigo de revisão pretende-se ilustrar como o modelo murino têm sido utilizado para a identificação de genes de resistência viral, e como estes podem funcionar como base para a descoberta de genes homólogos em outras espécies, na elucidação dos mecanismos de resistência, e em novos componentes da reunir todos os genes de resistência viral descobertos em murganhos

## INTRODUCTION

### *Virus-Host Interactions*

Human and animals differ widely in their responses to virus infections. Viruses may induce barely discernible symptoms in some of them and severe, life-threatening illness in others<sup>(1, 2, 3, 4, 21)</sup>. These differences reflect both the complex, intimate, and multifaceted nature of virus-host interactions and the variables that can influence these interactions. Viruses elicit a complex temporal sequence of stringently regulated innate and adaptive immune responses. These responses are essential for recovery, but they also contribute to disease symptoms. Optimal innate and adaptive immune responses are therefore balanced at a narrow interface between ineffectual and overly aggressive. Variables of age, gender, immune status, and genetic background influence the availability and form of the host metabolites on which viruses depend as well as the magnitude and effectiveness of the ensuing host response

<sup>(11)</sup>. Evidence is accumulating that genetic background is one of the major factors involved, thus, identifying genes that control the response to virus infection is a crucial step in elucidating how they might affect the pathophysiological processes underlying the severity of the disease induced<sup>(1, 2, 3, 4)</sup>.

### *The mouse model*

The large size and complexity of the human and domestic animals genome, have limited the identification and functional characterization of components of the innate immune system that play a critical role in front-line defence against invading virus. However, the advent of new technologies and resources, including the complete sequence of mammalian genomes, has had a dramatic impact on the genetic analysis of susceptibility to infections. Besides that, the study of the genomic organization and content of widely divergent vertebrate species has shown a remarkable degree of evolutionary

conservation, which enables the cross-species comparison and analysis of newly discovered genes.

Application of comparative genomics to host resistance will rapidly expand our understanding of human and domestic animals immune defence by facilitating the translation of knowledge acquired through the study of model organisms<sup>(9, 28, 38, 47)</sup>.

The mice have been for quite a long the model of choice<sup>(20, 21, 34)</sup>, more is known now about genetic control of resistance to viruses in mice than any other animal. They own this success to the low costs incurred, the existence of thousands of inbred strains, and the fact that the mouse is the species with the greatest density of known genetic markers, which facilitates the tracing of the alleles partly or wholly responsible for complex phenotypes<sup>(34)</sup>.

## VIRUS-RESISTANCE GENES

### *The Mx*

The mouse genome contains two related interferon-regulated genes, Mx1 and Mx2.

The Mx1 was both the first virus-resistance gene to be cloned and the first to be used to identify a human homologue, it encodes a type I interferon inducible nuclear protein that affords specific protection against influenza viruses. Only three mouse strains, A2G, SL/NiA and T9, express the antiviral form of protein; other mouse strains have deletions in the Mx1 gene that result in truncated proteins that are devoid of antiviral activity<sup>(45)</sup>.

The Mx2 gene is non-functional in all laboratory mice strains examined, since its open reading frame (ORF) is interrupted by an insertional mutation and a subsequent frameshift mutation. However, the Mx2 mRNA of cells from feral mouse strains NJL (*Mus musculus musculus*) and SPR (*Mus spretus*) that differs from that of the laboratory mouse strains tested is expressed upon interferon treatment and inhibits vesicular stomatitis virus, and hantavirus replication<sup>(30, 31)</sup>.

### *The Hv2*

Hv2, another mouse virus-resistance gene, controls resistance to mouse hepatitis virus

(MHV), a large-enveloped RNA virus that is distantly related to human enteric and respiratory coronaviruses. As natural pathogens of mice, MHV strains may cause either localized intestinal infections or generalized infections that emanate from the respiratory tract. All MHV strains appear to use members of the biliary glycoprotein subfamily as receptor proteins<sup>(19)</sup>. The principal receptors for MHV are several isoforms encoded by the biliary glycoprotein 1 (Bgp1) gene on the proximal end of mouse chromosome 7<sup>(39)</sup>.

The SJL inbred mouse strain, unlike almost all other inbred strains, is highly refractory to MHV infection. The Bgp1b (Hv2r) allele of SJL mice contains divergent sequences in the N-terminal virus-binding domain<sup>(33)</sup>, particularly in the critical residues 38-43<sup>(39)</sup>. The altered Bgp1b isoform remains a functional virus receptor, but its affinity for MHV is reduced by one to two orders of magnitude<sup>(37)</sup>.

### *The Flv*

Innate resistance to flavivirus, which includes human pathogens such as yellow fever, Dengue, and West Nile virus was demonstrated in mice in the 1920s as a monogenic autosomal dominant inheritance. The alleles that determined resistance and susceptibility were designated Flvr and Flvs, respectively<sup>(27)</sup>. Resistant mice are susceptible to infections with other types of viruses but are resistant to all flaviviruses<sup>(44)</sup>. Resistant mice can be infected by flaviviruses, but the virus titers in their tissues are lower by 1,000-10,000 times than those in the tissues of susceptible animals, and the spread of the infection in resistant mice is slower<sup>(17, 26)</sup>. Most commonly used inbred laboratory mouse strains have a homozygous flavivirus-susceptible genotype. Only the Det, BSVR, BRVR, CASA/Rk, CAST/Ei, MOLD/Rk, and PRI inbred strains have the resistant allele<sup>(7)</sup>.

### *The Cmv1*

Murine cytomegalovirus (MCMV) is a natural pathogen of mice that has been widely used as a model of human cytomegalovirus infection. Differences in susceptibility were observed among inbred mouse strains, being the

C57BL/6 resistant and BALB/c susceptible to MCMV infection <sup>(41)</sup>. MCMV titers in the spleens of susceptible mice were inversely correlated with their ability to mount an effective Natural Killer (NK) cell response <sup>(41, 42)</sup> suggesting an important role of those cells in the innate control of cytomegalovirus infection. Later on, this has been demonstrated in humans with a rare disorder characterized by complete absence of NK cells and in genetically deficient mice lacking NK cells or being depleted of those cells by treatment with anti-NK cell antibodies <sup>(7)</sup>. A single dominant locus, named *Cmv1*, located in the natural killer gene complex on mouse chromosome 6 controls the NK efficient response and consequently the resistance to MCMV infection in mice <sup>(18, 24, 41, 42, 43)</sup>. The alleles of the *Cmv1* locus can confer either susceptibility (*Cmv1s*, a recessive allele) or resistance (*Cmv1r*, a dominant allele) to MCMV.

### ***The Rmp***

Ectromelia virus, is a naturally occurring pathogen of mice and belongs to the Poxviridae family. Orthopoxviruses cause localized or generalized infections in a variety of species. Systemic infections which include smallpox (variola) in human beings are among the most lethal infections known <sup>(22, 23)</sup>.

Most inbred strains of mice are highly susceptible to the lethal effects of ectromelia virus infection, but C57BL and AKR strains are highly resistant <sup>(9, 13)</sup>. Susceptible strains succumb to the acute necrotizing effects of ectromelia virus on liver, spleen, and bone marrow, whereas resistant strains develop mild, transient inflammatory changes in target organs <sup>(12)</sup>.

Resistance to mousepox is inherited as a polygenic trait <sup>(14, 48)</sup>. At least four autosomal dominant genes, named *Rmp-1*, *Rmp-2*, *Rmp-3*, and *Rmp-4*, control resistance to mousepox in C57BL mice. The genes that control resistance to mousepox are of interest because they are dominant over ectromelia virus genes that encode virokinases and other virulence factors and because they may be some of the best examples of mouse genes that have responded

to selection pressure by a highly lethal naturally occurring virus.

### ***The Fv-1, Fv-4***

In 1957, Charlotte Friend described a novel retroviral disease in mice characterized by splenic enlargement, erythroleukemia, and death <sup>(25)</sup>. This rapidly progressive disease, now known as Friend disease, has provided a powerful tool for the study of multistage carcinogens and, most recently, has provided major insights into the molecular mechanisms of leukemia resistance.

A number of host genes have been identified that affect the susceptibility of mice to Friend virus-induced erythroleukemia. These genes can be divided into several groups based on their proposed mechanism of resistance: group 1: interference with retroviral infection (*Fv1*, *Fv4*); group 2: altered immune response (*Fv3*, *Rfv1*, *Rfv2*, *Rfv3*); and group 3: regulators of erythroid cell proliferation and differentiation (*Fv2*, *Fv5*, *Kit*, and *Mgf*) <sup>(5)</sup>.

In terms of virus-resistance genes only the first group, that consists of genes that dominantly interfere with the infection of target cells by the virus, will be mentioned. We have then, the *Fv1* gene that encodes a Gag-related protein, which interferes with the retroviral life cycle <sup>(6)</sup> and the *Fv4* gene that encodes a protein which blocks ecotropic viral receptors <sup>(29)</sup>.

Differences in susceptibility among mouse strains allowed the identification of those genes. In the *Fv1* gene, two alleles were discovered by this way: *Fv1n* and *Fv1b*. These alleles are able to block specific subclasses of MLV <sup>(8)</sup>. *Fv1n*, found in NIH Swiss mice, is able to block replication of B-tropic MLV while allowing replication of N-tropic virus, and *Fv1b*, found in BALB/c mice, acts vice versa. In the *Fv4* gene, the dominant allele was first described in a G strain of laboratory mouse, and subsequently, in several wild mouse populations in Japan, Southeast Asia and western North America <sup>(36, 46)</sup> as a single dominant gene for the resistance against MLV infection, independent of *Fv1*.

### ***H-2 class I genes***

Theiler's virus causes a persistent and demyelinating infection of the central nervous

system of the mouse, which is one of the best animal models to study multiple sclerosis.

All inbred mouse strains inoculated intracranially with Theiler's virus present with early encephalomyelitis. However, not all of them remain persistently infected. The C57BL/6 strain clear the infection after approximately 2 weeks. On the contrary, the susceptible strains (SJL and FVB/N) remain infected for life<sup>(15, 16, 40)</sup>.

The susceptibility of the mouse to persistent infection is multigenic. H2 class I genes have a major effect<sup>(32)</sup>. While most of the non-H2 susceptibility genes group control viral load during persistence by modulating the immune response. Among several non-H2 susceptibility loci, Tmevp3 appears to regulate the expression of important cytokines<sup>(10)</sup>.

### CONCLUSION

These examples illustrate how mouse-virus systems are being used to identify candidate virus-resistance genes. They provide the probes to detect functional homologues of resistance genes that are shared by rodent and other species. They delineate novel genetic mechanisms of resistance and highlight specific genetically complex and polymorphic components of the innate immune system as sources of variation in responses to viruses. Other benefits are likely to emerge as more mouse virus-resistance genes are identified and characterized. Clearly, not all details of virus-restriction pathways are conserved between mice and other species, but even where differences exist, functional similarities may be enough to guide genetic studies of resistance to viruses.

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